Biochemical and spectroscopic characterization of the high molecular weight cytochrome c from Desulfovibrio vulgaris Hildenborough expressed in Desulfovibrio desulfuricans G200.

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The gene of high molecular weight, multiheme cytochrome c (Hmc) from the sulfate-reducing bacterium Desulfovibrio vulgaris Hildenborough has been overexpressed in Desulfovibrio desulfuricans G200. The recombinant protein has been purified. Its molecular weight (65,600), amino acid composition, and NH2-terminal sequence were found to be identical to those of the wild-type protein. The recombinant protein has been spectroscopically characterized (optical spectrum, EPR, circular dichroism) and compared to the wild-type protein. We have found 16 hemes per molecule by iron analysis and the pyridine hemochrome test. Both high- and low-spin features were observed in the EPR spectrum. A detailed spin quantitation analysis indicates 1 or 2 high-spin hemes and 14 or 15 low-spin hemes per molecule. The redox potentials of the hemes determined by voltammetric techniques gave an average of three different values, 0, -100, and -250 mV (versus NHE), for the wild-type and the recombinant cytochrome. The low potential values are similar to the values observed for the bis(histidinyl) coordinated hemes of cytochrome c3. A comparison of the arrangement of heme binding sites and coordinated histidines in the amino acid sequences of cytochrome c3 and Hmc has shown that the latter contains four domains, three of which are complete c3-like domains, while the fourth represents an incomplete c3-like domain which may contain His-Met coordinated hemes. These data are in agreement with the detailed study of the number and types of hemes reported in this paper.

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